Clinical Infectious Diseases

BRIEF REPORT

Complement 5a Receptor Polymorphisms Are Associated With Panton-Valentine Leukocidin–positive *Staphylococcus aureus* Colonization in African Pygmies

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Panton-Valentine leukocidin (PVL) is common in African *Staphylococcus aureus* and can be associated with skin and soft tissue infection. PVL-positive *S. aureus* colonization is associated with a variant of complement receptor 5a, the cellular target of the lukS PVL subunit.

Keywords. *Staphylococcus aureus*; complement C5a; receptor; Panton-Valentine leukocidin; polymorphism.

Staphylococcus aureus is a major cause of skin and soft tissue infection (SSTI), particularly in Africa [1]. The incidence of SSTI is higher in African children than among children in the United States of a similar age group [2]. Several virulence factors are associated with SSTI, such as hemolysin α, phenol soluble modulins, the arginine catabolic mobile element, or the Panton-Valentine leukocidin (PVL) [3]. Sub-Saharan Africa is a PVL-endemic region, due to the high proportion of PVL-positive isolates among all *S. aureus* (17–74%) [2]. Although it is not entirely understood how PVL causes necrotizing infection, the high prevalence of PVL has a clinical impact and might fuel the SSTI burden in Africa.

PVL is a bi-component toxin comprised of lukS-PV and lukF-PV subunits. It exerts cytolysis after binding of lukS-PV to the complement 5a receptor I and II (C5aRI, C5aRII) and subsequent pore formation with lukF-PV [4].

It is unclear why PVL-positive *S. aureus* is widespread in Africa but rare in other geographical regions (eg, Europe). Specific determinants of the interaction of PVL with its host-cell target (C5aRI, C5aRII) could explain the difference in the prevalence. Since PVL is highly conserved, we hypothesized that C5aRI and C5aRII polymorphisms might be associated with a susceptibility of the host to PVL [5]. This study utilized a unique group of Babongo pygmies and investigated whether colonization of PVL-positive *S. aureus* is associated with C5aRI and C5aRII polymorphisms.

METHODS

Ethical Approval

The Comité d'Ethique Institutionnel of the Centre de Recherche Médicales de Lambaréné (CEI-CERMEL: 001/2011) granted ethical clearance for this study. The majority of the studied population was illiterate and/or only spoke a native tribal language. A local interpreter therefore explained the study procedures and we provided a short description of the study (in French), which was signed or fingerprinted by each participant, the investigator, and a local witness prior to any study-related procedures. The CEI-CERMEL approved the use of a documented oral informed consent [6].

Study Population

The study population consisted of Babongo or mixed Babongo/Mitsogo pygmies living in Waka National Park, Gabon. Most of them have no access to health care systems and were not registered locally. This particular ethnic minority group was repeatedly reported to have high colonization rates with PVL-positive *S. aureus* [6, 7]. Participants were included if they were Babongo or mixed Babongo and living in 1 of 6 Babongo settlements (ie, Village Tranquille, Tsibanga, Ossimba, Ndougou, Soga, Egouba). No exclusion criteria were applied. Participants were recruited in their villages based on availability. Considering a proportion of 60% PVL-positive isolates in carriers [6], the estimated sample size was 107 participants for each group (PVL-positive vs. PVL-negative carriers, $\alpha = .05$, power 80) which almost equals the total Babongo population (N = approx. 300) [8]. As a minimum requirement, we aimed to include one-third ($n \geq 100$) of the population.

Bacterial Culture

Each participant had separate nasal, pharyngeal, and wound swabs (if applicable) taken, which were cultured on Columbia blood agar and chromID *S. aureus* Elite agar (SAIDE, bioMérieux, Marcy l'Étoile, France). Species identification was done by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany) and the polymerase chain reaction detection of *S. aureus* protein A (*spa*). All isolates were *spa*-typed and 1 isolate per *spa* type was subjected to

Received 24 May 2018; editorial decision 24 July 2018; accepted 6 August 2018; published online September 6, 2018.

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Clinical Infectious Diseases® 2018;XX(XX):1–3

DOI: 10.1093/cid/ciy666

multilocus sequence typing. If a patient was colonized at multiple sites (nose, throat) with isolates that had the same *spa* type, antimicrobial resistance pattern, and PVL status (eg, PVL positive or negative), such that the swab profiles were identical, we only considered 1 isolate when reporting proportions (eg, sequence type [ST], PVL, antimicrobial resistance).

Genotyping of C5aRI and C5aRII

Bucccal mucosal swabs were taken from each participant using Forensic Swabs L (Sarstedt, Nümbrecht, Germany) to obtain human epithelial cells. Deoxyribonucleic acid (DNA) was extracted using the Quick-DNA Kit (Zymo Research, Irvine, California).

The C5aRI and C5aRII loci were Sanger-sequenced with pre-designed primer pairs covering the exome regions of *C5aRI* and *C5aRII* (Thermo Fisher Scientific, Wesel, Germany, Supplementary Table S1). Sequencing was done on a 3730 DNA Analyzer (Thermo Fisher Scientific) using NimaGen chemistry.

The sequences were mapped against the Human Genome 38 assembly to screen for single nucleotide polymorphisms (SNPs) using the University of California, Santa Cruz (UCSC) Genome Browser (https://genome.ucsc.edu/).

All statistical analyses were performed using "R" (version 3.1.2, http://www.r-project.org). Genotypic and allelic frequencies were analyzed by simple gene counting. The deviations from Hardy-Weinberg equilibrium were calculated by using a permutation test for each group. An association of C5aRI/II SNPs with (PVL-positive) *S. aureus* colonization was analyzed by binary logistic regression, adjusted for age and gender. The level of significance was set at $P < .05$.

RESULTS

The recruited Babongo (n = 107) had a median age of 13 years (range: 0.75–59) and a balanced ratio of females to males (50 vs 57, respectively). The overall colonization rate was 68.2% (n = 73), with a clear age-dependent colonization pattern (with the highest colonization in teenagers). Volunteers were colonized in the nose (n = 50), nose and throat (n = 15), nose and wound (n = 2), throat (n = 5), and throat and wound (n = 1). None of the wounds showed signs or symptoms of infection. Of the 73 carriers, 33 (45.2%) were colonized with PVL-positive *S. aureus* and 40 (54.8%) with a PVL-negative isolate.

After removing duplicate isolates, the final dataset consisted of 85 *S. aureus* isolates. The predominant ST were ST152 (n = 26, 73.1% PVL-positive), ST2788 (n = 20, 5% PVL-positive isolates), and ST6 (n = 8, no PVL-positive isolates). *Spa* types associated with ST152 were t127 (n = 7), t355 (n = 14), t2784 (n = 2), and t3636 (n = 3). Other STs were less diverse in terms of *spa* types, such as ST2788 (t189, n = 20) and ST6 (t1476, n = 8).

The isolates were resistant to penicillin (n = 64, 75.3%), trimethoprim/sulfamethoxazol (n = 14, 16.5%), and tetracycline (n = 11, 12.9%).

Human DNA was successfully extracted from all samples; the DNA yield was 4–112 ng/μL.

SNPs were detected in *C5aRI* (n = 12) and *C5aRII* (n = 7). None of the *C5aRI* SNPs (rs369189796, rs11670330, rs112996569, rs113016810, pos47269546, rs397897664, rs201148720, rs4467185, pos47320035, rs61733735, rs36028765, and rs11880097) or *C5aRII* SNPs (rs36046934, rs150649665, rs115216760, rs4482395, rs115584235, rs187635721, and rs112290646) were associated with *S. aureus* colonization (Supplementary Table S2). Interestingly, the pygmy population was monomorphic for 2 SNPs (DEL rs397897664 and rs4482395*G*). All genetic variants were in Hardy-Weinberg equilibrium, except for the variants rs112996569 C/T, rs113016810 G/A, and rs201148720 C/A.

In contrast, those carriers of PVL-positive *S. aureus* differed in 3 SNPs from those carriers with PVL-negative *S. aureus* (Table 1). The rs11880097*G* allele is a missense mutation (N279K) of the third extracellular domain of C5aRI that is associated with a protection against PVL-positive *S. aureus* colonization (Table 1). The 2 other SNPs are either a synonymous mutation (rs150649665) or an intron variant (rs187635721).

DISCUSSION

PVL binds to the C5a receptors of myeloid cells, culminating in cytolysis. We showed that 3 SNPs of C5aRI and II are associated with PVL-positive *S. aureus* colonization in a Gabonese pygmy population.

Although our sample size is small (n = 107), we included approximately one-third of the Babongo population, suggesting that our findings might hold true for the whole ethnic group. We found high *S. aureus* carrier rates (68.2%), exceeding those reported from Babongo recently (33%) [6]. The study was performed during the rainy season, which can be associated with higher colonization rates [9].

Of the 3 candidate SNPs associated with PVL-positive colonization (Table 1), rs11880097*G* confers a missense mutation of the third extracellular domain of C5aRI (N279K), and might therefore have a functional impact on the interaction of lukS-PV with C5aRI [4].

The SNP database includes allele frequencies of the "1000 genome consortium" (https://www.ncbi.nlm.nih.gov/SNP). In all groups of this database (Americans, Africans, East Asians, South Asians, and Europeans), rs11880097*G* is the minor allele. However, the frequency of rs11880097*G* in Africans is higher (6.4%) than in the other groups (0–2.5%). Constantly high exposure to *S. aureus* infections among Africans might be a positive selection pressure for rs11880097*G*, which was shown to be protective against PVL-positive *S. aureus* colonization [2]. In contrast, lower frequencies of *S. aureus* infections (or other agents) in Europeans might have exerted a lower selective pressure.

Recently, it was shown that not only the binding of lukS-PV to C5aRI, but also the interaction of lukF-PV with CD45, is critical for the susceptibility of myeloid cells to PVL [10]. This mechanism

Table 1. C5aR Genotypes and Allele Frequencies in Babongo Pygmies Being Colonized With *S. aureus* or PVL-positive *S. aureus*

C5aR SNPs		PVL-positive *S. aureus* Colonization, n (%)		Odds Ratio (95% CI)	*P* Value
		Yes (n = 33)	No (n = 40)		
rs11880097 T/G[a]	TT	26 (93)	21 (60)	Ref	
	GT	2 (7)	11 (31)	0.15 (0.03–0.8)	.011
	GG	0 (0)	3 (9)	NA	NA
	T	54 (96)	53 (76)	Ref	
	G	2 (4)	17 (24)	0.1 (0.03–0.5)	.001
rs150649665 C/A[b]	CC	31 (97)	30 (79)	Ref	
	CA	1 (3)	7 (18)	0.13 (0.02–1.2)	NS
	AA	0	1 (3)	0.3 (0.01–8.2)	
	C	63 (98)	67 (88)	Ref	
	A	1 (2)	9 (12)	0.12 (0.01–0.9)	.019
rs187635721 G/T[c]	GG	27 (93)	23 (72)	Ref	
	GT	2 (7)	6 (19)	0.3 (0.06–1.7)	NS
	TT	0	3 (9)	0.1 (0.006–2.5)	NS
	G	56 (90)	52 (81)	Ref	
	T	2 (10)	12 (19)	0.15 (0.03–0.7)	.008

Inheritance of mutant allele across all 3 C5aR loci (rs11880097, rs150649665, and rs187635721) predisposes the Babongo pygmies towards decreased susceptibility towards PVL-positive *S. aureus* colonization.

Abbreviations: C5aR, complement 5a receptor; CI, confidence interval; NS, not significant; PVL, Panton-Valentine leukocidin; Ref, reference; *S. aureus, Staphylococcus aureus*; SNP, single nucleotide polymorphisms.

[a]Sequencing failure in 10 participants.

[b]Sequencing failure in 3 participants.

[c]Sequencing failure in 12 participants.

was unknown while planning and conducting the study. However, variants of CD45 need to be analyzed to assess factors influencing the interaction of PVL with its cellular targets in future.

Our study has limitations. First, although a high proportion of PVL-positive *S. aureus* has been reported among Babongos, antimicrobial resistance rates increased and the clonal structure changed towards a predominance of ST152, compared to a previous study from 2009 [6]. Since the population structure does not seem to be stable, random effects of short-term changes should be considered. Second, we only obtained buccal swabs for DNA extraction, since the majority of Babongo would have not consented to blood samples. Poor DNA quality hampered the SNP analyses for a few participants. Since we were unable to draw blood samples, we did not assess whether rs11880097*G* altered the susceptibility of neutrophils to PVL.

In conclusion, the C5aRI SNP rs11880097 is associated with PVL-positive *S. aureus* colonization in an African pygmy population and affects the third extracellular domain of the receptor.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. F. S., A. W., and G. P. designed the study. A. F., A. S. A., and F. S. enrolled the participants and collected the data. A. W., M. S., and F. S. performed sequencing. A. W., M. S., F. S., P. G. K., B. L., P. F. Z., T. P. V., and G. P. analyzed and interpreted the data. F. S., A. W., and T. P. V. drafted the manuscript. All authors critically appraised the manuscript and approved the final version.

Acknowledgments. The authors thank Gerrit Randau for excellent technical assistance.

Financial support. This study was supported by institutional funds Institute of Medical Microbiology, University Hospital Münster).

Potential conflicts of interest. P. F. Z. received honoraria for lectures and advisory board meetings from Alexion Pharma Germany GmbH and VIFOR Fresenius Medical Care Renal Pharma. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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